ANNUAL PROGRESS REPORT

RCS MEDDH-288 (R1)

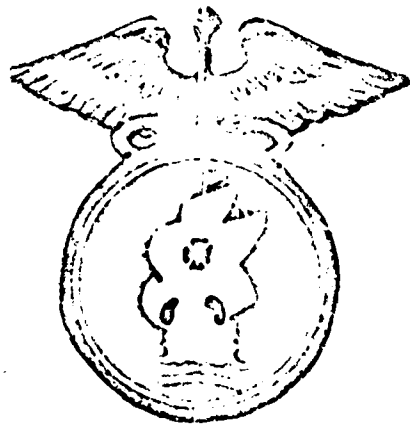
1 July 1972 - 30 June 1973

U.S. Army Medical Research Unit, Panama

Box 1809

APO New York 09826

July 1973



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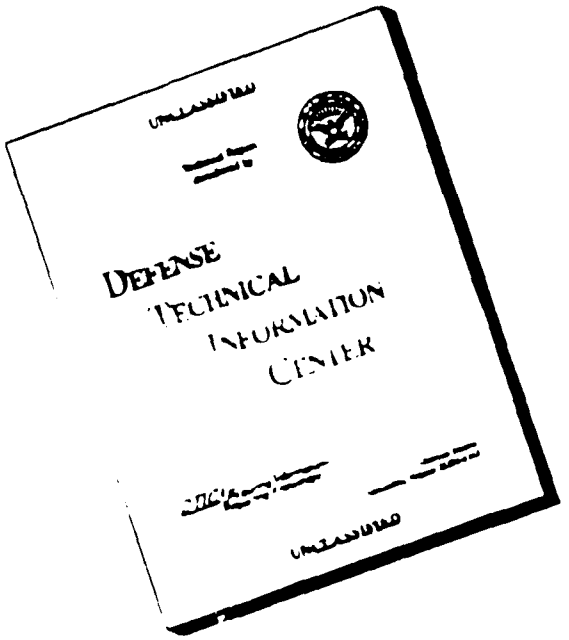

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ANNUAL PROGRESS REPORT

RCS MEDDH-288 (R1)

1 July 1972 - 30 June 1973

U.S. Army Medical Research Unit, Panama

Box 1809

APO New York 09826

Project No. 3A061102B71Q, Task 00, Work Units 110, 419, 421

July 1973

US ARMY MEDICAL RESEARCH UNIT - PANAMA

under technical direction of

WALTER REED ARMY INSTITUTE OF RESEARCH

an operating agency of

US ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND

is co-located with the

MIDDLE AMERICA RESEARCH UNIT
Ancon, Canal Zone

and administratively supported by

US ARMY FORCES SOUTHERN COMMAND

SUMMARY

The research program of USAMRU-P is devoted to the study of infectious diseases of military importance in Latin America. The investigations reported are primarily concerned with American leishmaniasis, a disease which continues to afflict US Forces personnel.

A study of the effect of chemotherapy on serum antibody was made to determine if serologic testing would be of value in judging treatment efficacy. Although few patients reverted to seronegativity, there was a demonstrable effect in most cases, and the rate of treatment failure was inversely correlated with diminution of titers. In one espundia case declining titers paralleled clinical improvement and an increase in titer accompanied an explosive recrudescence.

Follow-up of patients treated with metronidazole in the Canal Zone and Republic of Panama medical facilities demonstrated conclusively that this drug is of no value.

A vero cell experimental infection model for antileishmanial drug screening was developed which permits exposure of intracellular amastigotes to drug for up to 10 days. However, no effect on the parasites could be demonstrated with this system using a clinically effective drug, methyl glucamine antimoniate.

An incidental finding was made of the first human liver fluke (Amphimerus sp.) infection in Panama, which constitutes only the third report from the Western Hemisphere.

Normal hematologic values for the colonized cotton rats (Sigmodon hispidus) used for experimental infection studies were derived from monthly samples over a 6-13 month period.

The life-cycle of Angiostrongylus costaricensis was established in the laboratory and experimental infections studied in several species of wild rodents and 2 species of sub-human primates, which showed monkeys develop a disease picture resembling that of humans.

A 2 year study of breeding populations of phlebotomine sandflies and local environmental effects on these populations was completed. The species complement is divided into 2 major consociations, one dominated by anthropophilic species and the other by zoophilic species. The species complement includes 6 major species of which 4 are anthropophilic and have been implicated in transmission of American leishmaniasis. The effect of clinatologic, hydrologic, phytologic, and edaphic factors on sandfly distribution was studied.

Mass rearing techniques using standard laboratory glassware were developed which produced improved yields over traditional methods, with greatly reduced expenditure of man hours. Wild caught females were placed in culture preparations in which eggs were laid, hatched and immature stage development progressed with essentially no attention or care, which constitutes an important first step to colonization.

FORWARD

The research program of the U.S. Army Medical Research Unit, Panama is devoted to the study of infectious diseases and their vectors of military importance in Latin America.

The research projects were carried on under the following project and task number:

3A061102B71Q, Task 00, Work Units 110, 419, 421

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care", as promulgated by the Committee on the Guide for Laboratory Animal Resources National Academy of Sciences - National Research Council.

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.

TABLE OF CONTENTS

ANNUAL PROGRESS REPORT

PROJECT NO.	3A061102B71Q :	Communicable Disease and Immunology
TASK NO.	00 :	Ecology and Control of Disease Vectors and Reservoirs
WORK UNIT	421 :	Studies on Parasitic Infections in Latin America
REPORTING INSTALLATION	:	U.S. Army Medical Research Unit, Panama Box 1809 APO New York 09826
PERIOD COVERED BY REPORT	:	1 July 1972 - 30 June 1973
PRINCIPAL INVESTIGATOR	:	Bryce C. Walton, COL, MSC
ASSOCIATE INVESTIGATOR	:	Larry D. Hendricks, CPT, MSC
ASSISTANTS	:	Michael E. Hajduk, SFC, AMEDS Irma Arjona Julio J. Fuentes

REPORTS CONTROL SYMBOL: RCS-MEDDH-288 (R1)
SECURITY CLASSIFICATION: UNCLASSIFIED

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3. DATE PREVIOUS SUMMARY: 72 06 30	4. KIND OF CHANGE: R. Termination	5. SECURITY: U	6. [illegible]: U	7. RESEARCH: NA	8a. DISEN INVOLVED: NL	8b. SPECIFIC DATA CONTRACTOR ACCESS: YES NO	9. LEVEL OF SUM: A. WORK UNIT

10. NO. CODE	PROGRAM ELEMENT	PROJECT NO.	TASK AREA NUMBER	WORK UNIT NUMBER
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b. CONTRIBUTING	6[illegible]	3A0[illegible]B25	00	
c. CONTRIBUTING	6[illegible]	[illegible](T)		

11. TITLE (U) Studies of Parasitic Disease in Latin America (PM PZ)

12. SCIENTIFIC AND TECHNOLOGY CATEGORIES: 032600 Biology 031100 Microbiology 030900 Clinical Medicine

13. START DATE	14. EST. COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
64 07	73 06 30	DA	"9" - IN HOUSE

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18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR: PREV. FY 72	2	55
FISCAL YEAR: SUPPLMT 73	1 1/2	55

19. RESPONSIBLE SUB-ORGANIZATION
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NAME: Ackerman, Larry J., MAJ

21. GENERAL USE
"FOREIGN INTELLIGENCE CONSIDERED"

22. KEYWORDS (Provide EACH with Security Classification Code)
(U) Parasitic Disease (U) Leishmaniasis (U) Chemotherapy (U) Serology (U) Liver fluke (U) Leptospirosis

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) The acquisition of data concerning prevalence and distribution of parasitic diseases in Latin America; recognition of public health problems of actual or potential military importance; increase of knowledge of reservoirs and vectors; and improvement of diagnosis, treatment and control of these diseases.

24. (U) The effect of chemotherapy on serum antibodies of patients with primary cutaneous American leishmaniasis and with secondary mucocutaneous disease was studied with the Indirect Fluorescent Antibody test. The series included patients treated with cycloguanil pamoate, two different pentavalent antimonials, and with amphotericin B. The efficacy of metranidazole for the treatment of cutaneous American leishmaniasis was studied by follow-up of patients treated with this drug in 3 local medical facilities. A tissue culture drug screening system was tried as a means of in vitro determination of anti-leishmanial activity against intra-cellular amastigotes. The Hemolytic Leptospiral (HL) test was established to provide a tool to maintain surveillance for leptospiral infection among U.S. Forces personnel. Consultative services for diagnosis of unusual parasitic infections was provided to local medical facilities.

25. (U) A distinct response of serum antibodies to drug treatment was observed in most cases of leishmaniasis. Only a small percentage reverted to seronegativity, but some predictive value for recurrence of lesions was shown for serologic monitoring. Metranidazole was conclusively shown to be of no value in the treatment of American leishmaniasis. A tissue culture system which permitted exposure of infected cells to test compounds for up to 10 days was developed. However, no effect on intra-cellular amastigotes could be demonstrated with a clinically-effective drug. Leptospiral infections for a common source of exposure were confirmed for 3 men from one company of an Infantry Battalion. The first case of human liver-fluke infection from Panama

was diagnosed as an incidental finding.
This project has been transferred to Special Foreign Activities of Walter Reed Army Institute of Research and further progress will be reported in the Annual Technical report of WRAIR.

BODY OF REPORT

PROJECT NO. 3A061102B71Q : Communicable Diseases and Immunology

TASK NO. 00 : Ecology and Control of Disease Vectors and Reservoirs

WORK UNIT 421 : Studies of Parasitic Infections in Latin America

DESCRIPTION:

The objectives of this project are to determine, describe, and delimit the parasitic diseases of actual or porential military importance, and to develop improved methods for their diagnosis, treatment and prevention. The major proportion of the research effort was devoted to American leishmaniasis, a disease which continues to afflict US Forces personnel. A study of the effect of chemotherapy on serum antibody was made to determine if serologic testing would be of value in judging treatment efficacy. Although few patients reverted to seronegativity, there was a demonstrable effect in most cases, and lack of diminution of antibody level was shown to have some predictive value for treatment failure. Follow up of patients treated with metranidazole in Canal Zone and Republic of Panama medical facilities demonstrated unequivocally that this drug is of no value. In efforts to develop a tissue culture system for screening for antileishmanial activity, a Vero cell system was developed which allowed for drug exposure of infected cells for up to 10 days, but no effect on the intracellular parasites could be demonstrated in this system with a pentavalent antimonial compound which is clinically effective. In other diseases, the Hemolytic leptospirosis (HL) test was established to permit surveillance for this infection. An incidental finding of a human liver fluke (Amphimerus sp.) infection was of academic interest as the first such case in Panama, and only the third known locality in the Western Hemisphere.

PROGRESS:

In the past, effectiveness of chemotherapy of American leishmaniasis has been judged solely by the healing response of the lesion. However, it is well known that in some cases complete elimination of the parasite is not achieved in spite of clinical healing of the lesion, and recurrence or development of new lesions can occur. The new lesions may involve mucous membranes and can appear many years after the primary infection and cause severe facial mutilation, so the detection of residual viable parasites can be of extreme importance. It was considered that the effect of treatment on serum antibodies could add another

parameter to judging the effectiveness of drug treatment and a study was instituted to measure indirect fluorescent antibody (IFA) titers during and after treatment. Retrospective studies were done on sera of patients treated with Camolar[1] and Fuadin[2] and of patients treated more recently with Pentostam[3] and Glucantime[4].

There were detectable changes in serum antibodies following treatment in many cases. These data are presented in Figure 1. Increases in titer occurred only in cases of treatment failure demonstrated by the presence of parasites in culture or biopsy. Cases which showed no reduction in titer had a high rate of drug failures (9 of 16) while in those showing a titer reduction of even two-fold, the proportion was greatly reduced (8 of 27). With four-fold or greater reduction, there was only one failure in 23 cases. However, in the majority of cases antibodies persisted over long periods in spite of a prompt healing response and some reduction in titer. One of our patients has had positive IFA tests for 6 years, and we recently tested an individual who was treated with antimonials 34 years ago and still has antibody.

The reversion to negativity after treatment exhibited by some patients, Table 1, suggests the possibility that the persistence of antibody in the others might be due to the continued presence of viable parasites. Since the great majority of treated cases have persistent titers, this is a rather disturbing hypothesis because of the known propensity of L. braziliensis to remain latent for many years and then produce the mucosal lesions of espundia. In a few cases it has been possible to demonstrate the presence of viable parasites in clinically cured patients with persistent titers. One such patient was previously reported in the Annual Report for 1969. He had received three injections of Camolar which produced healing of his multiple lesions, but no reduction in titer. Three months later, he had reactivation of the lesions and parasites were seen in a biopsy. After a course of treatment with Fuadin, his lesions again disappeared, his antibody level diminished, and then fell below detectable levels. Another patient treated with Glucantime had prompt healing of a small primary ulcer on a finger, and a 4-fold reduction in antibody titer. In spite of this, 4 months after treatment he developed a new fungoid lesion on his elbow from which parasites were cultured. After excision of the lesion and retreatment, his antibody dropped below 1:8, but 1 1/2 months later it had returned to 1:16 (Figure 2). Although these examples are few in number, and most of the persistent-antibody patients have never shown any indication that they would relapse, they are nevertheless suggestive that drug treatment has not been completely effective for the majority of leishmaniasis cases in the Canal Zone and that the patients might retain latent infection which could result in mucosal lesions in the future.

The effect of treatment of advanced mucocutaneous leishmaniasis was studied in a Bolivian Army officer who presented with rapidly

progressing lesions after a history of unsuccessful treatment with sodium antimony tartrate, repeated courses of stibophen and 3 courses of cycloguanil pamoate over the preceding 2 1/2 years. The antibody response to pentavalent antimony and amphotericin B is demonstrated in Figure 3. The rapid diminution of antibody following Pentostam treatment paralled impressive clinical improvement. After amphotericin B therapy was terminated because of signs of renal toxicity, the patient experienced a sudden explosive recrudescence, and a serum sample demonstrated a rise in antibody titer.

A recent article in a leading journal reported success in the treatment of one case of American leishmaniasis with metranidazole (Flagyl®) and cited another article reporting some success with this drug against leishmaniasis in Mexico. In contrast to these reports, metranidazole failed in 4 out of 5 cases reported from Brazil. USAMRU-P recently had the opportunity of monitor treatment of 6 cases of primary cutaneous leishmaniasis acquired in Panama which were treated with this drug.

Six male patients with laboratory-confirmed leishmanial ulcers of 8-10 weeks evolution were treated; 4 in Gorgas Hospital, one at Gamboa Medical Clinic and one under the supervision of the Sanidad Militar of the Guardia Nacional in Panama City. Before treatment all patients had a general physical examination, chest roentgengram and base-line laboratory examinations consisting of urinalysis, complete blood count, platelet count, hemoglobin, hematocrit and SMA-12 battery. These laboratory tests were repeated 4 times at 10-day intervals, i.e., after the first treatment period, at the end of the rest period, and after 10 and 20 days of the second treatment period. Cultures were made from the margins of the lesions on the same schedule, and at 2- and 4-weeks after treatment was terminated.

Five patients between the ages of 19 and 52 years were found to be otherwise healthy and laboratory values were within normal limits. Patient #1, 14 years of age, had a neutropenia with a WBC count of 1,100/cu mm, polymorphonuclear neutrophils 15%, lymphocytes 79%, eosinophils 4%, and basophils 2%, a condition occasionally seen in children in Panama. This patient was hospitalized for diagnostic studies but no other abnormalities were encountered and the cause of these blood findings had not yet been determined.

Initial improvement of the appearance of the lesion was seen in all patients during the first treatment period. However, in patients no. 3 and 4 the improvement was temporary and by the end of the 10 day rest period (day 20) they had regressed to their original state. During the second treatment period improvement was much slower, even for those which did re-epithelialize.

Results of the cultures for leishmanial organisms are shown in Table 2. Only patient no. 2 showed consistently negative post-treatment cultures. Patient no. 6 at the conclusion of treatment appeared to have been successfully treated, but later developed a slight discoloration at the margin of the scar, and cultures inoculated at the 4-week follow-up grew promastigotes.

Some drug-attributable adverse effects were observed. Patient no. 1 exhibited a steady diminution of WBC count from 4,100/cu mm to 3,500/cu mm during the first period of treatment, rebounded slightly to 4,000/cu mm in the rest period, but again declined to 3,000/cu mm at day 30, whereupon treatment was terminated. Patient no. 2 likewise exhibited a steady diminution in the WBC from a baseline of 6,000/cu mm to 5,500/cu mm in the first treatment period, and to abnormally low values of 4,700/cu mm at day 30, and 4,300/cu mm at day 40, respectively. A weight loss of 8, 7, and 9 pounds was experienced by patients 2, 3 and 4 respectively.

The adult patients, nos. 2-6, were given 500 mg metranidazole 3 times daily for 10 days, a 10 day rest period was then followed by the same dose daily for 20 days. The additional 10 days beyond the standard regimen for amebiasis and trichomoniasis was decided upon because of steady improvement in the appearance, but incomplete healing of all lesions at the 20th treatment day, and the lack of any serious side affects due to the drug.

Patient no. 1, with a body weight of 41 kg, received 250 mg 3 times daily for 10 days, a 10 day rest period followed by another 10 days at 250 mg 3 times daily. The second treatment period was not extended as it was for the adult patients because of depression of his leucocyte count.

Successful treatment was achieved with only one patient. At the conclusion of the second treatment period two patients, nos. 2 and 6, had completely re-epithelialized the lesion and had no residual of the raised, erythymatous margin, but no. 6 subsequently was shown by culture to have residual viable leishmaniae.

The 5 treatment failures were subsequently treated with methyl glucamine antimoniate and complete healing with negative follow-up cultures was achieved with one course of 20 daily injections of 5 ml.

Although this series is small, these data demonstrate conclusively that metranidazole cannot be regarded as adequate chemotherapy for primary American leishmaniasis.

Lack of suitable experimental animal models for the screening of drugs for antileishmanial activity, and prior success in cultivating intra-cellular amastigotes of Panamanian strains of Leishmania in Vero

cells suggested the use of a tissue culture system for drug screening. A standardized inoculum of low-passage level promastigotes from blood agar media was found to give reproducible infections which could be maintained without overgrowth of the monolayer sufficient to interfere with accurate parasite counts through 10 days. A clinically effective drug, methyl glucamine antimoniate, was used for attempts to demonstrate drug effect, but no selective effect on parasites could be seen with this system, even when the exposure to the drug was extended to 10 days. At this juncture it was learned that a dog sarcoma-cell system developed by the Liverpool School of Tropical Medicine has been used for testing a small series of drugs, and although the system demonstrated the activity of other classes of compounds, no effect was seen on Leishmania mexicana by either tri- or penta-valent antimonial compounds. The Vero cell system is currently being evaluated with a series of non-antimonial drugs with known antiprotozoan properties.

In March 1971 a stool examination done by a USARSO laboratory on a local foodhandler revealed small operculated trematode eggs which were identified as Clonorchis sinensis and referred to USAMRU-P for confirmation. Repeated positive examinations over a period of several days established that this was not a spurious infection. Because C. sinensis is not known to occur in the Western Hemisphere, the foodhandler was again contacted for further investigation.

The patient was a 25 year old male Cuna Indian who had lived his entire life in the village of Mansukun, Comarca de San Blas in Panama. Two years after the original finding he was still passing the eggs. They were consistent in size and morphology with Amphimerus guayaquilensis previously reported from humans in one area in Ecuador and another in Colombia, and known to infect opossums and domestic cats in Panama.

Although he had no current complaints, he was admitted to Gorgas Hospital for physical and laboratory examinations. All findings were within normal limits except for a diffuse abnormal pattern on a liver scan.

Six clinically-suspect cases of leptospirosis among U.S. Forces personnel, including 3 from a common-source exposure among members of one company of an Infantry battalion, were confirmed by the HL test.

Publications

Walton, B.C., Brooks, W.H., & Arjona, I. 1972. The Indirect Fluorescent Antibody Test for Serodiagnosis of American Leishmaniasis. Am. J. Trop. Med. Hyg. 21 (3): 296-290.

Walton, B.C. & Yokogawa, M. 1972. Terrestrial Turbellarians as Pseudoparasites of Man. J. Parasit. 58 (3): 444-446.

Walton, B.C., Valverde, L. & Eguia, O. Onset of Espundia after Years of Occult Infection with Leishmania braziliensis. Am. J. Trop. Med. Hyg. In Press.

Table 2

Results of Culture for *Leishmania* Organisms
with Metranidazole Treatment

Patient No.	Pre Rx	Day of Treatment 10	20	30	40	Follow-up
1	+	+	+	-	ND	+ (2 wks post)
2	+	+	+	-	-	- (thru 4 wks)
3	+	+	+	+	+	none-retreated
4	+	+	+	+	+	none-retreated
5	+	+	+	+	+	none-retreated
6	+	+	+	+	-	+ (4 wks post)

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ANNUAL PROGRESS REPORT

PROJECT NO.	3A061102B71Q	:	Communicable Diseases and Immunology
TASK NO.	03	:	Entomology
WORK UNIT	110	:	Studies on Arthropods of Medical Importance in Latin America
REPORTING INSTALLATION		:	U.S. Army Medical Research Unit, Panama Box 1809 APO New York 09826
PERIOD COVERED BY REPORT		:	1 July 1972 - 30 June 1973
PRINCIPAL INVESTIGATOR		:	Louis C. Rutledge, MAJ, MSC

REPORTS CONTROL SYMBOL: RCS-MEDDH-288 (R1)
SECURITY CLASSIFICATION: UNCLASSIFIED

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	3. SUMMARY TYPE	4. REPORT CONTROL SYMBOL
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5. PERIOD COVERED	6. SUMMARY SCTY	7. WORK SECURITY	8. DOWNGRADING	9. DISP'N INSTR	10. SPECIFIC DATA (CONTRACTOR SCTY)	11. LEVEL OF SUM
72 06 30	U	U	NA	NL	30 YRS ☐ NO	A. WORK UNIT

12. NO. CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71P	00	119
b. CONTRIBUTING	61102A	3A061102B71P	03	
c. CONTRIBUTING	62703 13A(P)			

11. TITLE (Enter title with Security Classification Code)*

(U) Studies on Arthropods of Medical Importance in Latin America

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

005900 Environmental Biology 010100 Microbiology 003500 Clincal Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY			16. PERFORMANCE METHOD
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17. CONTRACT/GRANT Not applicable

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e. KIND OF AWARD f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING	72	1	21
FISCAL YEAR CURRENT	73	1	21

19. RESPONSIBLE DOD ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER: [redacted]

ASSOCIATE INVESTIGATORS

NAME: Ellenwood, Dennis A.

NAME:

21. GENERAL USE

"FOREIGN INTELLIGENCE NOT CONSIDERED"

22. KEY WORDS (Precede EACH with Security Classification Code)

(U) Phlebotomine (U) Lutzomyia (U) Sandflies (U) leishmaniasis

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) Evaluation of the disease vector potential of and vector control possibilities for selected arthropods of medical importance in Central America.

24. (U) Studies focus on phlebotomine sandflies as vectors of cutaneous leishmaniasis. The methods and concepts of systems ecology are utilized to acquire quantitative data on breeding habits, feeding preferences, prevalence, etc. and to interpret the data in terms of human disease and disease vector control.

25. (U) A 2 year study of breeding populations of phlebotomine sandflies (Diptera; Psychodidae) in the open forest floor was completed. The species complement includes 6 major species, of which 4 are anthropophilic, and have been implicated in the transmission of leishmaniasis. The dominant species was Lutzomyia trapidoi which occupies 85% of the area of the forest floor at an average population density of 8.3 per m^2 of soil. In all species, a population maximum occurs in early rainy season, and in all except 2 spp. a second peak occurs in late dry season. Distribution of forest litter regulated species distribution, L. trapidoi was greater on steep hillsides with light litter deposits, while L. panamensis, L. gomezi, L. pessoana and L. insolita were more common on hilltops and prominences with heavy, stable deposits. Similarly, certain large trees and vines produce litter which favors certain species. Present information indicates American leishmaniasis occurs in restricted microfoci, but this study shows vectors are distributed throughout the forest area, indicating that incidence of leishmaniasis is limited by some factor other than vectors, and probably is dependant upon suitable vertebrate reservoir hosts. Mass rearing of sandflies in the laboratory in petri dishes utilizing soil substrates and leaf litter for food of larvae hatched from eggs of wild-caught sandflies was employed. Yields several times greater than with traditional methods were obtained with greatly reduced

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

man-hours of effort, although the results were variable. Even with shortcoming, the method offer promise of providing sufficient numbers of adult sandflies for colonization trials and disease transmission studies.

This project has been transferred to Special Foreign Activities of Walter Reed Army Institute of Research and further progress will be reported in the Annual Technical report of WRAIR.

BODY OF REPORT

PROJECT NO.	3A061102B71P :	Communicable Diseases and Immunology
TASK NO.	03 :	Ecology and Control of Disease Vectors and Reservoirs
WORK UNIT	110 :	Studies on Arthropods of Medical Importance in Latin America

DESCRIPTION:

The objective of this study is to evaluate the disease vector potential of and vector control possibilities for selected arthropods of medical importance in Central America. Current studies focus on the phlebotomine sandflies as vectors of American leishmaniasis.

APPROACH.

The basic biology and field ecology of species of medical interest are investigated through an integrated program of laboratory and field studies. The results are interpreted in terms of the epidemiology of human disease and disease vector control.

PROGRESS:

Sandfly breeding sites. This study was initiated in FY 1971 and completed in FY 1973. Breeding populations of phlebotomine sandflies in the soil of the open forest floor were studied in a rain forest near Gamboa, Canal Zone. Sandfly collections were made with soil emergence traps from 43 selected sites within the study area. Supporting studies of local environmental effects on breeding populations were made concurrently with the sandfly collections. The general results of these studies are summarized in the following paragraphs.

The species complement of the forest floor breeding habitat includes 6 major species, of which 4 are anthropophilic and 2 are zoophilic (Table 1). The species Lutzomyia gomezi and L. rorotaensis had not been previously recovered from natural breeding sites. The species L. insolita had not been previously recovered from the forest floor habitat. All 4 of the anthropophilic species collected have been implicated in the transmission of American leishmaniasis in other studies. The species L. pessoana is the major day-biting anthropophilic species of the study area.

This study represents the first application of the methods of population ecology to immature populations of New World sandflies. Breeding populations were found to be thinly dispersed throughout the entire forest floor habitat (Table 1). The dominant species was L. trapidoi, which occupies some 85% of the area of the forest floor at an average population density of 8.3 per m^2 of forest soil.

In all species a population maximum occurs in the early rainy season, and in all except L. rorotaensis and L. insolita a second population maximum occurs in the early dry season. Similarly, in all species a population minimum occurs in the late rainy season, and in all except L. rorotaensis and L. insolita a second population minimum occurs in the late dry season. In brief, population maxima tend to occur when soil moisture conditions are moderate, and population minima tend to occur when soil moisture conditions are extreme, either wet or dry.

Supporting studies demonstrated that the forest litter tends to be removed by erosion from steep slopes and lower elevations and to cumulate on gentle slopes and higher elevations. This pattern of distribution of the forest litter was reflected in the spatial pattern of immature sandfly populations. Populations of L. trapidoi were greater in streamside and hillside regions of the study area, while populations of L. panamensis, L. gomezi, L. pessoana and L. insolita were greater in hilltop regions of the study area. However, only L. panamensis and L. pessoana were collected from alluvial litter at the edge of the stream.

Similarly, certain large trees and lianas were found to produce exceptionally deep and extensive deposits of litter. Populations of immature sandflies were generally greater in this situation than elsewhere within the general forest area. Only L. trapidoi and L. panamensis were collected from palm litter.

The practical implications of the foregoing results seem clear. Since several vector species breed continuously throughout the forest, they are not amenable to control through selective treatment of restricted areas or at particular seasons. Effective control will depend on development of methods for treating large areas of the forest efficiently and cheaply. In the meantime, the use of personal protective measures should be emphasized.

Present information indicates that American leishmaniasis occurs in restricted microfoci within the general forest area. On the other hand, our study of sandfly breeding sites indicate that several vector species breed continuously throughout the forest area. This discrepancy in the patterns of distribution of the parasite and its vectors suggests that the incidence of leishmaniasis is limited by some factor other than the availability of suitable vectors. The limiting factor may be, instead, the availability of suitable vertebrate reservoir hosts.

Canal Zone Sandfly Associations.

An analytical study of available records of adult sandfly light-trap collections from the Canal Zone was initiated and completed during FY 1973. These collections, representing 24 locations within the Zone, were made by elements of the U.S. Army, Southern Command, and the Middle America Research Unit over the period 1968 to 1972. A total of 551 collections records, representing 10,070 sandflies of 37 species, were analyzed.

It was concluded that the collections analyzed represented 2 basic sandfly consociations dominated, in one case, by the zoophilic species L. carpenteri, L. triramula and L. composi and, in the other, by the anthropophilic species L. gomezi and L. panamensis and the zoophilic species L. dysponeta. Both consociations were highly variable, and a number of disparate and intergrading species compositions were present in the collections analyzed. Each consociation occurs in varying terrain and on both the Atlantic and Pacific slopes of the Isthmus. They occur successively in certain locations, but one or the other is prevalent throughout the year in other locations. The anthropophilic consociation was more prevalent than the zoophilic consociation.

It seems probable that those areas and seasons in which the anthropophilic species predominate are a greater hazard to human health than others. Our data suggest that the sandfly species compositions of differing areas and seasons are regulated primarily by patterns of rainfall and drainage and that the anthropophilic consociation is favored by relatively wet situations. More detailed study of sandfly associations in the field will be required for confirmation of this point.

Sandfly Ecology on the Pacific Slope

Previous studies of sandfly ecology and population dynamics in Panama have been made on the Atlantic slope of the Isthmus. However, ecological conditions are quite different on the Pacific slope, where the majority of infections with American leishmaniasis in U.S. personnel are acquired. A study of sandfly ecology at Empire Range (the area of most active transmission on the Pacific slope) was initiated in November, 1972.

Adult sandflies are collected with light-traps at 20-meter intervals along a 200-meter transect twice weekly. The transect samples 3 biotopes characteristic of the area: (1) A grassy, disturbed area intersected by a road and by rights-of-way for a power line and a pipeline. (2) An adjoining secondary forest of chiefly hardwoods, (3) A primary forest including numerous palm trees. The data obtained include population density data by species, biotope and season.

A total of 24 species have been collected in the study area (Table 2). The ubiquitous zoophilic species L. carrenteri and the important anthropophilic species L. panamensis together make up 75% of the species composition. Other anthropophilic species collected in the study area include L. olmeca, L. sanguinaria, L. trapidoi, L. gomezi, L. [illegible]ana, and L. ylephiletrix.

A summary of the collections by transect station is given in Table 3. The population trend is toward increasing densities deeper in the forest. Sandfly populations were least in the disturbed area (stations 1 to 4), intermediate in the secondary forest (stations 5 to 8) and greatest in the primary forest (stations 9 to 11). Population microfoci were observed at station 3 in the disturbed area and at station 6 in the secondary forest. The region of station 9 in the primary forest evidently represents a "pocket" in the sandfly population distribution. Studies of environmental conditions prevailing at the several stations are being made in an effort to explain the occurrence of such microfoci and pockets.

Sandfly collections were continuous throughout the dry season (December through April) (Table 4) although the dry season of 1972-3 was one of the driest on record and the Pacific slope normally receives only about 1/2 the amount of rainfall received by the Atlantic slope. The population maximum occurred in the early dry season (January 1973, 9.7 flies/trap/night) and the population minimum occurred in the late dry season (April 1973, 1.2 flies/trap/night). The timing of these 2 events agrees fully with results obtained on the Atlantic slope in a prior study (see above). However, at Empire Range an intercalary population maximum was also observed (March 1973, 8.3 flies/trap/night). Since this resurgence occurred two months after that of January 1973, it may be due to population brook-effects. Data from laboratory rearing trials and from soil emergence trap collections indicate that the normal generation time is about 8 weeks for most species.

The species L. carrenteri was predominant throughout the dry season (December 1972 to April 1973) in the Empire Range collections. The anthropophilic species L. panamensis has been predominant in wet season months to date (November 1972, May-June 1973). Populations of L. panamensis declined steadily during the dry season, and this species was outnumbered by the common zoophilic species L. trinidadensis during the late dry season (February to April 1973).

The foregoing fluctuations in sandfly population densities and exchanges of dominance between zoophilic and anthropophilic species agree fully with the results obtained in our analysis of Canal Zone sandfly associations (see above). Their effects on the epidemiological pattern of sylvan leishmaniasis are uncertain, but they are undoubtedly important. We may expect to find, for example, that the pattern of

incidence of leishmaniasis in animal populations is continually shifting and changing within the forest area.

Mass-Rearing of Sandflies

Experimental studies of the transmission of leishmaniasis are presently hampered by the lack of large numbers of sandflies for experimental use. In addition to problems connected with survival, mating, blood-feeding and oviposition in the adult stage, the laboratory production of sandflies presents unique problems in connection with rearing the immature stages. Several features of sandfly biology contribute to the difficulty of mass-rearing the immature stages: (1) The eggs are produced in relatively small numbers (about 30 per clutch in most species). (2) The immature stages (egg, larva and pupa) are exceptionally sensitive to extremes of moisture and temperature. (3) The period of development is exceptionally long, usually 6 to 12 weeks. (4) The immature stages develop in a milieu of decomposing organic materials which is difficult to control and standardize.

Sandflies are traditionally reared in unglazed earthenware pots placed in trays of water. The method is cumbersome and requires constant attention to water level, larval food supply and fungal growth. Consequently, it is necessary to expend large amounts of manpower in the care of the colony to rear large numbers of sandflies by this method. The ideal culture would be self-regulating with regard to moisture content, food supply and fungal growth for the entire period of growth and development of the immature stages (6 to 12 weeks). Experiments toward this end were initiated during FY 1973.

In initial experiments 58 single-brood cultures (chiefly of L. trapidoi) were prepared as follows: Clay soil was homogenized with water to a pasty consistency in a Waring blendor. Standard disposable plastic Petri dishes (9 cm diam. x 1.2 cm deep) were filled with this substrate to a depth of $\pm$ 6 mm. The clay substrate was then covered with a thin layer of leaf fragments prepared by comminuting dried leaves in a Waring blendor. A single blood-fed, wild-caught female sandfly was then introduced into each Petri dish and confined with the cover. These were allowed to oviposit and die within the dishes, and their progeny to develop without further care. The rationale for this kind of culture is as follows: (1) It simulates conditions in natural breeding sites in the forest (see above). (2) The moisture-binding properties of clay soils give long-term retention of moisture. (3) The decomposing leaf fragments provide food for the entire period of growth and development of the sandfly larvae.

Sandfly larvae failed to appear in 27 (47%) of the 58 cultures prepared. This loss is referable to shortcomings of survival, mating and oviposition in the adult stage and of hatching in the egg stage.

The efficacy of the method is best judged on the basis of the 32 cultures in which larvae appeared. A total of 240 adult sandflies were obtained from the latter cultures. This total represents an average yield of 7.5 sandflies per culture. The potential of this method, if refined and standardized, is indicated by the maximum yield obtained, 24 sandflies in culture no. 48. It was noted in these trials that the more moist cultures were more productive of sandflies and less affected by fungal overgrowth.

In a second series of trials, an attempt was made to improve on the foregoing results by increasing initial moisture content and replacing a part of the moisture loss. Due to the non-availability of L. trapidoi, L. gomezi was substituted in 15 of 148 cultures in this series. In addition, to greater amount of water initially, 1/2 of the moisture lost through evaporation in the first 5 weeks of culture was replaced with a pipette during the sixth week of culture.

The foregoing modification of technique was unsuccessful. A total of 117 (79%) of the cultures were larva-negative. The yield of adult sandflies from the 31 larva-positive cultures amounted to 86, for an average of 2.8 sandflies per culture. The maximum yield was 9 sandflies in culture no. 173 (L. trapidoi).

The cause of the higher proportion of larva-negative cultures observed in this experiment is not definitely known. It is believed that the low yield obtained in larva-positive cultures was due to adverse temperature and humidity conditions in the laboratory environment. The cultures were kept in a non-air-conditioned room at ambient temperature and humidity. The first experiment, yielding 7.5 sandflies per positive culture, was conducted during the wet season (August to November 1972). The second experiment, yielding 2.8 sandflies per positive culture, was conducted chiefly in the dry season (January to June 1973). Although no measurements were taken in the first experiment, the rate of evaporation from the cultures was noticeably less than in the second experiment. In the second experiment, moisture losses were determined on a weekly basis for 7 sample cultures (to which no water was added in the 6th week). The average weight of the cultures at the time of preparation (excluding the weight of the Petri dish) was 58.0 gm, of which 34.9 gm (60%) was water. After 6 weeks, an average of 15.7 gm of water (45% of the original content) had been lost, and after 12 weeks, an average of 27.9 gm of water (80% of the original content) had been lost. It seems clear from our results that this rate of evaporation is unsatisfactory and that it can not be remedied by a one-time replenishment.

A primary purpose of this investigation is to develop a culture method which does not depend on sophisticated, extraneous equipment (air conditioners, humidifiers, incubators, etc.) for success. If

such a method can be developed, it will be effective under both controlled and uncontrolled conditions, as circumstances permit. Accordingly, current experiments continue to be directed toward development of a reliable, self-regulating culture system.

A total of 234 sandfly cultures of 12 types are currently in progress (Table 5). Evaporation has been reduced in cultures utilizing a dry substrate by reducing the surface area/volume ratio and the ventilation of the culture. The principle of the plaster substrate method is similar to that of the unglazed earthenware pot method, but evaporation is reduced by the plastic walls and cap of the container. The results of these and subsequent trials are expected to lead to a method of mass-rearing of the immature stages which will provide sufficient numbers of adult sandflies for colonization trials and/or disease transmission studies.

Publications

Rutledge, L.D., R.A. Ward and D.E. Hayes. 1973. Plasmodium cynomolgi: The comparative infectiousness of individual rhesus monkeys. Exper. Parasitol. 33; 120-126.

Rutledge, L.D., R.A. Ward and R.M. Buckwalter. 1973. Plasmodium spp. Dispersion of malarial oocyst populations in anopheline and culicine mosquitoes. Exper. Parasitol. (In press).

Christensen, H.A., and L.C. Rutledge. 1973. Two new synonyms in the genus Lutzomyia (Diptera, Psychodidae). J. Med Ent. 10:314.

Ward, R.A., D.E. Hayes, S.C. Hembree, L.C. Rutledge, S.J. Anderson and A.J. Johnson. 1972. Infectivity of Plasmodium falciparium gametocytes from Aotus trivirgatus to anopheline mosquitoes.

Table 1. Population data for sandfly species breeding on the open forest floor in Panama.

	Mean Population Density (per m^2)	Areal Prevalence (%)
*Lutzomyia trapidoi**	8.3	85
*L. panamensis**	3.3	65
*L. gomezi**	3.0	40
*L. pessoana**	2.8	60
L. rorotaensis	2.7	70
L. insolita	2.3	70
Totals	22.4	100%

*Anthropophilic species.

Table 2. Sandfly collections at the Empire Range study area by species as of 30 June 1973.

Species	No. Flies Caught	%
L. carpenteri	727	48.05
L. olmeca bicolor	30	1.98
L. panamensis	405	26.77
L. barretoi	17	1.12
L. vespertilionis	8	.53
L. trinkmula	57	3.77
L. trinidadensis	41	2.71
L. sanguinaria	15	.99
L. trapidoi	68	4.49
L. runoides	5	.33
L. gomezi	25	1.65
L. ovallesi	2	.13
L. aclydifera	3	.20
L. pessoana	14	.93
L. vesicifera	11	.73
L. camposi	64	4.23
L. ylephiletrix	2	.13
L. nordestina	9	.59
L. serrana	2	.13
L. cruciata	3	.20
L. dysponeta	2	.12
Brumptomyia spp.	1	.07
L. shannoni	1	.07
L. dasymera	1	.07
	1513	99.99

Table 3. Sandfly collections at the Empire Range study area by transect station as of 30 June 1973

Station	No. Times Trapped	No. Flies caught	Mean No. of Flies/trap/night
1	27	6	0.22
2	20	10	0.50
3	25	24	0.96
4	26	9	0.35
5	29	150	5.17
6	27	219	8.11
7	18	120	6.67
8	25	146	5.84
9	16	42	2.63
10	24	289	12.04
11	20	498	24.90
		1513	

Table 4. Sandfly collections at the Empire Range Study area by month and principal species as of 30 June 1973.

November 1972	152 flies	24 Trapping Nights	6.3 flies/trap/night	
	L. panamensis	60 flies	39.47%	
	L. carpenteri	59 flies	38.82%	
December 1972	206 flies	28 Trapping Nights	7.4 flies/trap/night	
	L. carpenteri	83 flies	40.29%	
	L. panamensis	64 flies	31.07%	
January 1973	339 flies	34 Trapping Nights	9.7 flies/trap/night	
	L. carpenteri	175 flies	51.62%	
	L. panamensis	58 flies	17.11%	
February 1973	149 flies	31 Trapping Nights	4.8 flies/trap/night	
	L. carpenteri	103 flies	69.13%	
	L. trinidadensis	10 flies	6.71%	
March 1973	297 flies	36 Trapping Nights	8.3 flies/trap/night	
	L. carpenteri	248 flies	83.50%	
	L. trinidadensis	9 flies	3.03%	
April 1973	44 flies	36 Trapping Nights	1.2 flies/trap/night	
	L. carpenteri	26 flies	59.09%	
	L. trinidadensis	4 flies	9.09%	
May 1973	93 flies	35 Trapping Nights	2.7 flies/trap/night	
	L. panamensis	48 flies	51.61%	
	L. carpenteri	17 flies	18.28%	
June 1973	233 flies	32 Trapping Nights	7.3 flies/trap/night	
	L. panamensis	174 flies	74.68%	
	L. carpenteri	16 flies	6.87%	
TOTALS	1513 flies		5.7 flies/trap/night	
	L. carpenteri	48.05%		
	L. panamensis	26.77%		
	L. trinidadensis	2.71%		

Table 5. Sandfly cultures in progress on 30 June 1973.

No. of cultures	Sandfly			Container			
	Species	No.	Stage	Material	Dimensions[1]	Ventilation[2]	Substrate[3]
100	L. trapidoi	1	Adult	Plastic	2.3x8.4	-	Clay
15	L. gomezi	1	Adult	Plastic	2.3x8.4	-	Clay
70	L. trapidoi	1	Adult	Glass	5.4x2.8	1 Perforation	Clay
30	L. gomezi	1	Adult	Glass	5.4x2.8	1 Perforation	Clay
5	L. trapidoi	1	Adult	Plastic	6.5x5.0	5 Perforations	Clay
1	L. sanguinaria	5	Adult	Plastic	7.0x4.3	1 Perforation	Clay
1	L. trapidoi	96	Adult	Plastic	8.5x10.5	1.5 cm screen	Plaster
2	L. trapidoi	500	Egg	Plastic	8.5x10.5	1.5 cm screen	Plaster
2	L. gomezi	500	Egg	Plastic	8.5x10.5	1.5 cm screen	Plaster
2	L. gomezi	200	Egg	Plastic	8.5x10.5	1.5 cm screen	Plaster
4	L. trapidoi	200	Egg	Plastic	6.0x6.0	1.5 cm screen	Plaster
2	L. gomezi	200	Egg	Plastic	6.0x6.0	1.5 cm screen	Plaster
234							

1. Dimensions are given as depth x diameter in cm.

2. The provision for ventilation of the culture is either a 1.5 cm diam. nylon screen in the cap or 1 or more perforations in the cap.

3. The plaster substrate is 9 parts plaster of Paris: 1 part activated charcoal (V/V). The container is perforated on the bottom and kept in a tray of water.

ANNUAL PROGRESS REPORT

PROJECT NO.	3A061102B71Q :	Communicable Diseases and Immunology
TASK NO.	00 :	Ecology and Control of Disease Vectors and Reservoirs
WORK UNIT	419 :	Pathology and Pathogenesis of Naturally Occurring Infections in Latin America
REPORTING INSTALLATION	:	U.S. Army Medical Research Unit, Panama Box 1809 APO New York 09826
PERIOD COVERED BY REPORT	:	1 July 1972 - 30 June 1973
PRINICIPAL INVESTIGATOR	:	Larry J. Ackerman, MAJ, VC
ASSISTANTS	:	John A. Williams Emilia P. Hoyos Michael E. Hajduk, SFC, AMEDS

REPORTS CONTROL SYMBOL: RCS-SGRD-288 (R1)

SECURITY CLASSIFICATION: UNCLASSIFIED

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
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3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISB'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
	H. Termination	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71Q	00	419
b. CONTRIBUTING	62110A	3A062110A 806	00	
c. CONTRIBUTING	CARDS 114 (f)			

11. TITLE (Precede with Security Classification Code) (U) Studies on Pathology and Pathogenesis of Naturally Occurring Infections in Latin America (PA, BZ, BL)

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17. CONTRACT GRANT Not applicable
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	CURRENT 73	1	25

19. RESPONSIBLE DOD ORGANIZATION
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23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) 1. To gather information concerning the reservoir status, modes of transmission and pathogenesis of infectious diseases produced by important anthropozoonotic and amphixenotic agents. 2. To furnish pathology support to professional investigators of the MARU staff. 3. To furnish animal pathology consultation to other military medical facilities for indigenous anthropozoonoses monitoring.

24. (U) Necropsies of non-human vertebrates are performed, supplemented with standard and special histopathology techniques as well as clinical pathology techniques. Searches are made of possible natural reservoir wild animals, domesticated mammals and invertebrate hosts. The pathogenesis of selected tropical diseases are studied in experimental animals.

25. (U) 72--7-73-06- The pathology service continued to support the studies of Venezuelan equine encephalomyelitis (VEE), yellow fever, Bolivian hemorrhagic fever and other natural and experimentally induced diseases in laboratory animals. Diagnostic support continued to be given to Air Force and Army Veterinarians servicing the two Canal Zone zoos, as well as examinations of wild animals for diseases indigenous to the area. Concentrated investigation has been on (1) normal developmental hematological changes in Sigmodon hispidus (2) development of animal models for the study of leishmaniasis and (3) incidence and histopathological changes of rodents infected with Angiostrongylus costaricensis and in the non-human primates, Cebus capucinus and Ateles geoffroyi.

This project has been transferred to Special Foreign Activities of Walter Reed Army Institute of Research and further progress will be reported in the Annual Technical Report of WRAIR.

*Available to contractors upon originator's approval

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

BODY OF REPORT

PROJECT No. 3A061102B71Q : Communicable Diseases and Immunology

TASK NO. 00 : Ecology and Control of Disease Vectors and Reservoirs

WORK UNIT 419 : Pathology and Pathogenesis of Naturally Occurring Infections in Latin America

DESCRIPTION:

The objective of this study is to gain information on the reservoir status, modes of transmission and pathogenesis of diseases of animals occurring in the area. Necropsies of subhuman animals supplemented by routine and special histopathologic examinations were performed in monitoring and characterizing a variety of disease changes in wild, domesticated and laboratory animals. Experimental infections and clinical pathology techniques are utilized whenever necessary for complete characterization of a disease process.

PROGRESS:

During FY 73 347 animals were examined and 181 accessions made. Part of the case material resulted from the support and pathology services rendered to other investigators of USAMRU-P and MARU and to the Air Force and Army Veterinarians servicing the Canal Zone and Republic of Panama. The remainder of case material resulted from independent research conducted by the Pathology section.

Diagnostic consultation support service included: 22 Calomys callosus for the Bolivian hemorrhagic fever studies, 10 horses for the Venezuelan equine encephalomyelitis studies, 4 Aotus trivirgatus monkeys in support of Yellow fever studies, numerous mice for tests for human Coxsacki virus infection, and over 200 additional animals for disease surveillance.

Angiostrongylus costaricensis

During this fiscal year, a laboratory cycle of Angiostrongylus costaricensis was established and maintained using the natural rodent host, Sigmodon hispidus and a slug Vaginulus sp. as the molluscan intermediate host. Important segments of the life cycle were elucidated. Fourteen days after the feces of infected Sigmodon were fed to slugs, a molt of second stage larvae into third stage larvae occured. At 21 days, the third stage larvae were extracted by pepsin-Hcl acid digestion of the infected slugs. Laboratory reared Sigmodon were fed these infectious larvae by gavage. Larvae penetrated the

mucosa of the small intestine and/or cecum, migrated to the submucosa of the same organ and molted within these tissues. Immature adult worms migrated to a regional artery and 6 days following infection, the young adults penetrated the artery and continued to mature within the lumen. Between 14 and 21 days oviposition occurred and by 28 days first stage larvae were passed in the feces of the infected rodents.

In order to gain information regarding the natural reservoir hosts, several species of wild caught and laboratory raised rodents and 2 species of nonhuman primates were tested to determine their susceptibility to infection with A. costaricensis. The results of these studies are summarized in Table 1.

These studies demonstrated several interesting facts. (1) When exposed to 3rd stage larvae Sigmodon hispidus has yielded as high as 70% recovery of adult worms in branches of the cranial mesenteric artery, aorta and even in the left ventricle of the heart. (2) Rattus norvegicus can serve as a laboratory model for this infection. (3) The wild rodents Zygodontomys microtinus, Proechimys semispinosus, Nyctomys sumichrasti, and Marmosa sp. do not pass 1st stage larvae and thus do not perpetuate the lifecycle of this parasite. (4) The hamster and guinea pig react similarly and do not perpetuate the lifecycle. (4) A massive single dose of 3rd stage of A. costaricensis in a susceptible host can result in mechanical blockage of branches of the cranial mesenteric artery with subsequent infarction of the lower intestinal tract and/or cecum resulting in peritonitis and/or death. (6) Two species of non-human primates, Cebus capucinus and Ateles geoffroyi are able to perpetuate the cycle of A. costaricensis following experimental infection. The route of migration of the worms and the time sequence for maturation and oviposition closely paralleled that of Sigmodon hispidus. Both species developed granulomatous inflammatory reactions similar to those reported in human infections; and 4 of the infected monkeys subsequently died of this disease. (7) Some monkeys (but not all) of both species passed 1st stage larvae in the feces 28 days after infection; a finding which has not yet been demonstrated in human infections.

Exo-antigen was obtained from adult worms maintained up to 14 days in medium 199 containing 2% penicillin-streptomycin and 0.5% amphotericin B. Crude antigen from adult A. costaricensis and the exo-antigen were utilized for serological testing of non-human primates. Thus far, ouchterlony, complement-fixation, and hemagglutination tests have not yielded a reliable test system. Skin tests administered to infected Cebus capucinus and Ateles geoffroyi monkeys have negative results regardless of whether or not the monkey was passing first stage A. costaricensis larvae in its feces.

Hematology of Sigmodon hispidus

Prior to initiation of studies of infection with Leishmania and agents of other tropical diseases in Sigmodon it was deemed necessary to establish average or standard hematologic values for the animals of our colony in order to interpret disease-induced changes.

Hemograms were obtained on 36 Sigmodon (21 males and 15 females) once a month for 6 months. Additional hemograms were determined on 19 of these animals (13 males and 6 females) for an additional period of 1-7 months. Each hemogram consisted of hematocrit, hemoglobin concentration, total erythrocyte, reticulocyte and leukocytes counts and leukocyte differential. Absolute leukocyte values and erythrocyte indices were calculated utilizing standard formulas.

Blood samples (0.5 ml) were collected during light ether anesthesia from the ophthalmic venous plexus using sterilized non-heparinized Pasteur-type pipettes. Thin blood films were prepared and the remaining blood transferred to a tube containing EDTA anticoagulant (1.5 mg EDTA/ml of whole blood).

Hematocrit was estimated using the microhematocrit method and hemoglobin concentration was determined by the cyanmethemoglobin method.

Total erythrocyte and leukocyte counts were done by the standard methods with a model A Coulter Counter, using dilutions of 1:500,000 and 1:500 respectively. The diluent was isotonic saline and the stromalysing agent was LYSES (Coulter). The machine was calibrated for Sigmodon hispidus blood utilizing the following settings: aperture, 6; erythrocyte threshold, 10; and leukocyte threshold, 20. Each count was made in triplicate and an average recorded for the sample.

Thin blood films were air dried and stained with Wrights'-Giemsa stain (buffered pH 6.8). Two hundred leukocytes were differentiated utilizing the cellular morphology criteria of Hepworth[1] including the distinction between large and small lymphocytes.

Reticulocytes were enumerated per 1000 erythrocytes according to the method of Johns[2] on smears of blood vitally stained with brilliant cresyl blue and counter stained with Wrights' stain.

The erythrocytic parameters are presented in Tables 2-3. During the entire 13 months of sequential sampling, there were no significant differences between male and female S. hispidus for any given age group. The erythrocyte numbers and hematocrit values did not change significantly over the 13 month period. There was however, a significant regression (F test: $P<.001$) of the hemoglobin concentration with

age in both male and female Sigmodon. During the first two months of life both male and female Sigmodon had a sharp reduction in the number of reticulocytes. After 2 months of age, these values progressed in a linear fashion throughout the next 11 months of life.

The erythrocyte parameters in our study are in close agreement with the erythrocyte and hematocrit values reported by Dunaway and Lewis[3] and with the hematocrit and hemoglobin values reported by Forman[4]. Our values, however, are higher than the values reported by Hepworth[1] for both "conditioned" and "stressed" wild caught Sigmodon; including reticulocyte values above 1%.

The leukocyte values are presented in Tables 4-5. During the 13 month period of investigation there was no significant difference between the leukocytes of males versus females or among age groups.

All the Sigmodon had a significantly higher number of lymphocytes than of neutrophils with the small lymphocytes outnumbering the large type. These findings are in agreement with Hepworth and others in classifying Sigmodon as one of the "lymphocytic-type" rodents (5). Other points of agreement with Hepworth's Sigmodon population are similar eosinophil and basophil counts and lower number of neutrophils in female versus males. Points of difference are lower monocyte counts and higher total leukocyte counts of our animals when compared to the "conditioned" wild-caught animals of Hepworth.

Even though previous results are based on single or double samples, the differences between individual results stress the importance of evaluating blood parameters of study groups thus avoiding differences which can arise due to geographic location or environmental conditions.

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TABLE 1
SUMMARY OF
ANGIOSTRONGYLUS COSTARICENSIS
INFECTION IN SEVERAL SPECIES OF ANIMALS

GENUS SPECIES	(COMMON NAME)	NUMBER INOCULATED	POSITIVE FECALS	NEGATIVE FECALS	NEGATIVE ADULTS	MORTALITY RATE
ATELES GEOFFROYI	(SPIDER MONKEY	11	10	1	0	2
CAVIA PORCELLUS	(GUINEA PIG)	3	0	3	3	0
CEBUS CAPUCINUS	(WHITE FACE MONKEY)	7	6	1	0	2
LIOMYS ADSPERSUS	(SPINY POCKET MOUSE)	1	1	0	0	1
MARMOSA SP.	(MOUSE OPOSSUM)	2	0	2	2	0
MESOCRITETUS AURATUS	(HAMSTER)	2	0	2	0	2
MUS MUSCULUS	(MOUSE)	10	10	0	0	2
NYCTOMYS SUMICHRASTI	(VESPER RAT)	2	0	2	0	2
PROECHIMYS SEMISPINOSUS	(SPINY RAT)	2	0	2	2	1
RATTUS NORVEGICUS	(NORWAY RAT)	4	4	0	0	0
SIGMODON HISPIDUS	(COTTON RAT)	18	18	0	0	5
ZYGODONTOMYS MICROTINUS	(CANE MOUSE)	16	0	16	8	0
	TOTAL	78	49	29	15	16

TABLE 2

ERYTHROCYTE CONSTITUENTS OF SIGMODON HISPIDUS

PARAMETER	AGE IN MONTHS					
	1	2	3	4	5	6
ERYTHROCYTES ($X10^6/mm^3$)	5.67± 0.82* (3.70-8.90)	6.11± 0.48 (4.94-7.23)	6.16± 0.52 (5.32-7.59)	6.23± 0.66 (4.86-7.87)	5.88± 0.53 (4.80-7.14)	5.97± 0.64 (4.40-7.25)
HEMATOCRIT (%)	42.47± 4.56 (31.00-52.00)	47.81± 2.97 (40.00-55.00)	46.75± 2.66 (39.00-52.00)	46.75± 2.86 (40.50-55.00)	44.05± 3.78 (37.00-51.50)	45.65± 4.34 (34.00-56.00)
HEMOGLOBIN (g/100ml)	14.69± 2.00 (10.00-18.50)	14.18± 1.31 (11.80-17.20)	13.69± 1.54 (11.50-17.50)	14.15± 1.51 (10.00-16.80)	13.51± 1.51 (10.80-16.50)	12.88± 1.36 (7.80-15.50)
MCV (μ)	75.92± 10.02 (46.07-102.70)	78.70± 7.29 (64.52-99.19)	76.31± 5.71 (64.56-86.67)	75.63± 6.75 (63.53-90.73)	75.42± 8.78 (58.23-100.00)	77.09± 9.83 (55.46-109.09)
MCH (μμg)	26.28± 4.20 (15.17-36.49)	23.36± 2.87 (18.07-31.98)	22.26± 2.35 (18.42-26.66)	22.91± 3.06 (14.84-29.42)	23.09± 2.78 (17.93-27.08)	21.68± 2.29 (16.46-26.22)
MCHC (%)	34.64± 3.47 (27.78-43.78)	29.70± 2.63 (24.08-36.60)	29.30± 3.48 (23.96-35.56)	30.34± 3.38 (21.28-35.11)	30.66± 2.03 (27.08-34.38)	28.39± 3.31 (16.25-35.23)
RETICULOCYTES (#/100 RBC)	10.58± 6.97 (2.10-25.60)	3.87± 1.44 (1.20-6.30)	3.20± 1.50 (0.70-6.90)	3.33± 1.28 (1.40-6.50)	4.27± 1.18 (1.80-8.70)	3.66± 1.92 (1.20-12.80)
No. of Animals	36	36	36	36	36	36

* Mean± S.D.
(Range)

TABLE 3

ERYTHROCYTE CONSTITUENTS OF SIGMODON HISPIDUS

PARAMETERS	AGE IN MONTHS						
	7	8	9	10	11	12	13
ERYTHROCYTES ($x10^6/mm^3$)	5.56± 0.67* (4.26-7.06)	5.73± 0.75 (3.82-6.54)	6.09± 0.75 (5.14-8.22)	5.90± 0.59 (4.54-6.88)	6.06± 0.48 (5.13-6.82)	6.04± 0.41 (5.43-6.97)	5.33± 0.74 (4.20-6.21)
HEMATOCRIT (%)	44.00± 2.96 (38.50-49.00)	45.50± 2.49 (41.00-50.00)	45.90± 3.29 (42.50-55.50)	43.30± 2.71 (38.00-48.50)	44.13± 4.26 (38.00-51.00)	43.83± 3.41 (39.00-50.00)	41.88± 4.69 (31.00-48.00)
HEMOGLOBIN (g/100ml)	13.47± 0.65 (12.50-14.50)	13.05± 1.63 (7.50-14.80)	12.83± 1.19 (9.80-14.50)	11.21± 1.85 (8.50-14.30)	12.00± 0.98 (10.30-13.30)	12.84± 1.21 (10.00-14.30)	11.87± 1.49 (9.30-14.30)
MCV (μ)	80.02± 9.77 (62.00-105.63)	80.68± 11.28 (71.21-117.80)	75.90± 4.97 (67.52-83.66)	73.81± 6.34 (63.23-85.90)	72.89± 5.99 (62.09-81.77)	72.71± 5.70 (63.93-85.64)	79.41± 11.35 (63.92-103.57)
MCH (μμg)	24.54± 3.15 (20.13-34.04)	23.14± 4.07 (13.00-33.51)	21.32± 3.09 (14.71-25.38)	18.96± 2.12 (14.97-22.31)	19.86± 1.82 (17.16-22.78)	21.31± 2.71 (17.04-24.86)	22.69± 4.48 (15.37-32.14)
MCHC (%)	30.70± 1.67 (27.14-33.75)	28.77± 3.75 (15.46-32.44)	28.05± 3.00 (20.21-31.76)	25.86± 3.66 (21.25-31.43)	27.35± 2.68 (22.55-32.82)	29.32± 2.18 (25.00-33.33)	28.48± 3.03 (22.41-33.33)
RETICULOCYTE	3.79± 0.67 (2.80-5.10)	4.91± ** (1.50-22.80)	4.33± ** (1.20-19.70)	4.98± 4.59 (1.60-20.00)	2.92± 2.27 (0.60-8.60)	3.20± 0.93 (1.80-4.40)	3.60± 1.17 (2.20-6.30)
No. of animals	19	17	15	15	12	12	12

*Mean ± S.D. (Range)

**Skewed Distribution of Values Rendered Calculated S.D. Invalid for Population.

TABLE 4

LEUKOCYTE CONSTITUENTS OF SIGMODON HISPIDUS

PARAMETER	AGE IN MONTHS					
	1	2	3	4	5	6
LEUKOCYTES ($x10^3/mm^3$)	9.05± 2.30 (5.03-15.10)	7.58± 2.66 (3.50-14.20)	7.75± 1.68 (5.10-12.10)	7.89± 2.13 (4.00-12.90)	7.75± 1.78 (3.30-12.80)	8.38± 2.39 (4.70-16.90)
NEUTROPHILS *($x10^2/mm^3$)	9.91± 7.27 (0.87-35.49)	12.40± 8.95 (1.56-40.12)	17.33± 15.00 (1.78-76.23)	17.16± 10.41 (2.12-50.31)	23.88± 8.59 (7.26-53.53)	20.05± 10.61 (7.05-55.77)
BANDS ($x10^2/mm^3$)	0.64± *** (0.00-4.96)	0.64± *** (0.00-4.52)	0.76± *** (0.00-2.70)	0.62± *** (0.00-3.13)	0.27± *** (0.00-1.92)	0.72± 0.61 (0.00-2.18)
LYMPH SMALL ($x10^2/mm^3$)	72.70± 21.19 (40.55-124.79)	57.67± 27.28 (18.48-132.06)	53.02± 15.14 (23.40-95.76)	53.71± 18.84 (0.00-91.25)	49.92± 13.72 (24.75-93.44)	56.96± 16.59 (31.78-98.87)
LYMPH LARGE ($x10^2/mm^3$)	1.71± *** (0.00-8.76)	1.54± *** (0.00-4.84)	1.97± 1.40 (0.00-6.30)	1.92± 1.32 (0.00-5.10)	0.25± *** (0.00-1.74)	1.37± *** (0.00-8.45)
MONOCYTES ($x10^2/mm^3$)	1.08± 0.98 (0.00-3.65)	0.93± 0.83 (0.00-3.08)	0.94± 0.57 (0.00-2.13)	1.16± 0.95 (0.00-3.87)	0.43± *** (0.00-1.76)	0.88± *** (0.00-5.58)
EOSINOPHILS ($x10^2/mm^3$)	4.41± 2.48 (0.00-9.31	2.63± 1.53 (0.50-6.00)	3.11± 1.94 (0.00-9.00)	3.48± 2.05 (0.80-10.40)	2.77± 1.82 (0.00-7.70)	4.34± 2.89 (1.08-14.50)
BASOPHILS ($x10^2/mm^3$)	0.04± *** (0.00-0.52)	0.04± *** (0.00-0.57)	0.17± *** (0.00-1.19)	0.05± *** (0.00-0.65)	0.00	0.07± *** (0.00-0.54)
No. of Animals	36	36	36	36	36	36

*Absolute count

** Mean± S.D. (Range)

*** Skewed Distribution of Values Rendered Calculated S.D. Invalid for Population.

TABLE 5

LEUKOCYTE CONSTITUENTS OF SICALOLOX HISPIDUS

PARAMETER	AGE IN MONTHS						
LEUKOCYTES ($x10^3/mm^3$)	6.68± 1.06** (5.00-9.40)	7.20± 2.04 (3.70-10.80)	7.23± 2.00 (4.50-10.70)	8.47± 2.70 (4.30-13.40)	6.78± 2.87 (3.10-11.60)	7.45± 1.27 (5.70-9.80)	7.43± 1.27 (5.20-9.80)
NEUTROPHILS *($x10^2/mm^3$)	16.45± 7.15 (4.35-28.35)	13.14± 11.89 (1.80-43.74)	16.37± 10.33 (6.49-43.17)	17.83± 14.12 (3.64-59.59)	19.36± 15.78 (2.64-63.22)	17.51± 6.27 (9.69-30.55)	18.40± 5.85 (11.56-28.11)
BANDS ($x10^2/mm^3$)	0.28± *** (0.00-1.90)	0.52± *** (0.00-1.90)	0.46± *** (0.00-1.61)	0.50± 0.49 (0.00-1.50)	0.11± *** (0.00-0.49)	0.75± 0.55 (0.00-1.80)	0.60± *** (0.00-2.58)
LYMPH SMALL ($x10^2/mm^3$)	46.30± 7.94 (33.75-64.86)	53.80± 16.38 (22.76-88.80)	51.04± 12.24 (30.38-75.44)	59.60± 18.78 (34.19-102.51)	44.21± 18.93 (22.36-78.80)	51.90± 11.38 (33.32-73.01)	50.93± 12.09 (35.88-76.44)
LYMPH LARGE ($x10^2/mm^3$)	0.42± *** (0.00-1.52)	0.89± 0.52 (0.00-1.78)	0.94± 0.45 (0.32-2.08)	1.40± 1.22 (0.00-3.60)	1.10± *** (0.00-4.06)	0.73± 0.71 (0.00-2.35)	0.57± ** (0.00-2.35)
MONOCYTES ($x10^2/mm^3$)	0.52± 0.44 (0.00-1.45)	0.38± *** (0.00-2.35)	0.49± 0.45 (0.00-1.61)	1.05± 0.95 (0.00-3.00)	0.33± *** (0.00-1.64)	0.88± 0.47 (0.00-1.54)	0.48± *** (0.00-1.78)
EOSINOPHILS ($x10^2/mm^3$)	2.79± 1.65 (0.74 -7.60	2.23± 1.16 (0.00-3.78)	3.06± 0.91 (1.83-4.85)	4.42± 3.02 (0.65-12.00)	2.77± 2.24 (0.16-6.70)	2.74± 1.49 (0.00-5.39)	3.36± 1.23 (1.56-5.10)
BASOPHILS ($x10^2/mm^3$)	0.02± *** (0.00-0.38)	0.01± *** (0.00-0.19)	0.00	0.04± *** (0.00-0.54)	0.00	0.00	0.00
No. of Animals	19	17	15	15	12	12	12

*Absolute count

** Mean± S.C. (Range)

*** Skewed Distribution of Values Rendered Calculated S.D. Invalid For Population.

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13. ABSTRACT

The research program of USAMRU-P is devoted to the study of infectious diseases military importance in Latin America. The investigations reported are primarily concerned with American leishmaniasis, a disease which continues to afflict US Forces personnel.

A study of the effect of chemotherapy on serum antibody was made to determine if serologic testing would be of value in judging treatment efficacy. Although few patients reverted to seronegativity, there was a demonstrable effect in most cases, and the rate of treatment failure was inversely correlated with diminution of titers. In one espundia case declining titers paralled clinical improvement and an increase in titer accompanied an explosive recrudescence.

Follow-up of patients treated with metranidazole in the Canal Zone and Republic of Panama medical facilities demonstrated conclusively that this drug is of no value.

A vero cell experimental infection model for antileishmanial drug screening was developed which permits exposure of intracellular amastigotes to drug for up to 10 days. However, no effect on the parasites could be demonstrated with this system using a clinically effective drug, methyl glucamine antimoniate.

An incidental finding was made of the first human liver fluke (Amphimerus sp.) infection in Panama, which constitutes only the third report from the Western Hemisphere.

Normal hematologic values for the colonized cotton rats (Sigmodon hispidus) used for experimental infection studies were derived from monthly samples over a 6-13 month period.

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The life cycle of Angiostrongylus costaricensis was established in the laboratory and experimental infections studied in several species of wild rodents and 2 species of sub-human primates, which showed monkeys develop a disease picture resembling that of humans.

A 2 year study of breeding populations of phlebotomine sandflies and local environmental effects on these populations was completed. The species complement is divided into 2 major consociations, one dominated by anthropophilic species and the other by zoophilic species. The species complement includes 6 major species of which 4 are anthfopophilic and have been implicated in transmission of American leishmaniasis. The effect of clinatologic, hydrologic, phytologic, and edaphic factors on sandfly distribution was studied.

Mass rearing techniques using standard laboratory glassware were developed which produced improved yields over traditional methods, with greatly reduced expenditure of man hours. Wild caught females were placed in culture preparations in which eggs were laid, hatched and immature stage development progressed with essentially no attention or care, which constitutes an important first step to colonization.

14. KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
Leishmania, leishmaniasis, cutaneous, espundia, chemotherapy, serology, vector, Lutzomyia phlebotomine sandflies, Angiostrongylus costaricensis, pathogenesis, Amphimerus, liver fluke, Panama.						